REMARKS

Claims 1-26 are pending in the present application. Claims 2-19, 21, 22 and 24 and 25 are withdrawn as being directed to non-elected inventions. Claims 20 and 23 are amended herein for clarity and to more particularly define the invention. In addition, withdrawn claim 1 is amended herein in order to put it into condition for rejoinder upon allowance of the product claims. Support for these amendments is found in the language of the original claims and throughout the specification, as set forth below. It is believed that no new matter is added by these amendments and their entry and consideration are respectfully requested. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

I. Claim Objections.

The Action states that claims 20 and 23 are objected to. Action, page 2. The Action requests that the term "sequence" be inserted after "miRNA" and before "endogenous" in claim 20, lines 5-6, and in claim 23, line 6. *Id.*

Claims 20 and 23 are amended herein as requested to recite the term "sequence" between "miRNA" and "endogenous," thereby obviating this objection. Accordingly, applicants respectfully request its withdrawal.

II. Rejection under 35 U.S.C. §112.

A. Enablement.

The Action states that claims 20, 23 and 26 stand rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the enablement requirement. Action, page 2. The Action alleges that the specification does not reasonably provide enablement for a plant cell or plant transformed with an miRNA precursor construct comprising any plant miRNA precursor sequence which is modified to contain a non-native (exogenous and heterologous) sequence replacing the native miRNA sequence in said plant miRNA precursor sequence. Action, page 3. This rejection is respectfully traversed.

The test of enablement under 35 USC §112, first paragraph, is not whether any experimentation is necessary but rather is whether one skilled in the art could make or use the invention from the disclosure in the patent coupled with information known in the art without undue experimentation. *See, e.g.,* MPEP 2164.01. Further, it is well settled that "a patent need not teach, and preferably omits, what is well known in the art" *Id.* In addition, in order to make a rejection, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *See* MPEP 2164.04. It is also specifically noted that a specification which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the claimed subject matter must be taken as in compliance with the enablement requirement unless there is a reason to doubt the objective truth of the statements contained therein.

In determining whether or not the enablement requirement is satisfied in any particular case and whether any necessary experimentation is undue, reference is typically made to the factual inquiries specified in *In re Wands*, and provided in MPEP § 2164.01(a). The inquiries include the following:

(A) The breadth of the claims;

(B) The existence of working examples;

(C) The nature of the invention;

(D) The state of the prior art;

(E) The level of one of ordinary skill;

(F) The level of predictability in the art;

(G) The amount of direction provided by the inventor; and

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

In this case, when the above factors are evaluated, it is respectfully submitted that the enablement requirement is clearly satisfied. Hence, each of these factors is discussed in greater detail below.

(A) The breadth of the claims.

Claims 20 and 23 as presented herein recite an miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, said plant miRNA precursor having an miRNA sequence incorporated into the plant miRNA precursor, wherein (a) the miRNA is modified as compared with the miRNA sequence endogenous to said isolated plant miRNA precursor, the modifications maintaining the length of the endogenous miRNA; and (b) the isolated plant miRNA precursor is modified to correspond to the modifications made in the miRNA, the modifications to the plant miRNA precursor maintaining the secondary structure of the plant miRNA precursor including double strandedness and any mismatches, and further wherein, the modified miRNA is complementary to and hybridizes with a target nucleotide sequence within said plant whereby the expression of the target sequence is reduced. Support for these amendments is found throughout the specification, for example, at least, on page 5, lines 19-26; on page 31, lines 25-28; on page 32, lines 8-24; and on page 33, lines 27-31. No new matter is added by these amendments.

Thus, the claims as presented herein are well focused and encompass miRNA precursor constructs comprising an isolated plant miRNA precursor in which the endogenous miRNA is modified so that it is complementary to and hybridizes with a target nucleotide sequence of interest while at the same time such modifications maintain the length of the endogenous miRNA sequence. Further, the plant miRNA precursor is described as modified in the region opposite of the miRNA so as to maintain double strandedness including any mismatches, thus retaining the secondary structure of the plant miRNA precursor. In view of the features recited in the claims of the present invention, it is respectfully submitted that the claims are well focused and their breadth is clearly defined within the disclosure of the specification. Thus, it is submitted that this factor weighs in favor of the applicants.

(B) The existence of working examples.

It is well settled that the presence of working examples is not required to

satisfy the enablement requirement. *In re Strahilevtz*, 212 USPQ 561, 563 (CCPA 1982); MPEP 2164.02. Furthermore, the courts have noted that the specification need not contain an example if the invention is otherwise disclosed in such a manner that one skilled in the art will be able to practice it without an undue experimentation. *See, e.g., In re Borkowski*, 422 F.2d 904, 908, 164 USPQ 642, 645 (CCPA 1970).

Applicants respectfully submit that the present invention is disclosed in such a manner that one skilled in the art is able to practice it without undue experimentation. This contention is supported by the numerous after-filing examples applicants have previously provided showing that the invention works as claimed (See, for example, Vaucheret et al., *Genes & Development* 18: 1187-1197 (2004); Schwab et al., *Plant Cell* 18: 1121-1133 (2006); Alvarez et al., *Plant Cell* 18: 1134-1151 (2006); and Nui et al., *Nat Biotechnol.* 24(11):1420-8 (2006); copies provided previously). Further examples evidencing that the present invention works as described include Choi et al. (*Development* 134: 1931-1941 (2007) and Qu et al. (*J. Virol.* 81: 6690-6699 (2007)) (copies of each of these references are attached at Tab C).

(C). The nature of the invention, (D) the state of the prior art and (E) the relative skill of those in the art. Applicants respectfully submit that these three factors weigh in favor of the applicants.

The nature of the invention becomes the backdrop to determine the state of the art and the level of skill possessed by one skilled in the art (M.P.E.P. § 2164.05(a)). Applicants' invention pertains to a plant stably transformed with a miRNA precursor construct, which comprises a plant miRNA precursor in which the endogenous miRNA sequence of the plant miRNA precursor is modified as compared to the endogenous miRNA sequence. The techniques utilized for making the miRNA constructs of the present invention are standard nucleic acid manipulations that were well known and routine in the art at the time the application was filed.

Clearly, the level of skill in this field is high, and those in the field have ready access to the prior art, which describes not only the many known plant miRNA precursors and the common structural elements that define them but also the techniques needed to make and use the present invention as claimed. For instance, the technique for modifying the miRNA sequence endogenous to the plant miRNA precursor utilizes the well-known process of compensating nucleotide base changes in which the primary nucleotide composition of a nucleotide sequence is modified while retaining any secondary structure that is present in the original nucleotide sequence. This technique is discussed in the textbook *Biochemistry* (Berg, J.M., J.L. Tymocko, L Stryer, *Biochemistry* 5th Ed., W.H. Freeman and Co. (2002), section 7.3.5, Figure 7.19). Further, as an example of its use, applicants enclose a copy of a 1996 journal publication by Ma and Matthews, which shows the use of compensating base changes to determine the importance of a RNA stem in adenovirus function (Ma and Mathews, RNA 2: 937-951(1996). In Ma and Matthews, the effects of various compensating and noncompensating base changes on the structure of the stem were also determined experimentally. Compensating base changes do not alter the structure of the stem.

Thus, applicants respectfully submit that the three factors, the nature of the invention, the state of the prior art and the relative skill of those in the art, each weigh in favor of applicants.

(F) The level of predictability in the art, (G) the amount of direction provided by the inventor and (H) the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

Applicants respectfully submit that these three factors also weigh in favor of the applicants.

Although the biological sciences are generally categorized as "unpredictable," the courts have long and repeatedly emphasized that the issue is not predictability *per se*, but the type of work and experimentation acceptable in the particular field, or fields, of the invention. For example, in *In re Angstadt,* the Court of Customs and

Patent Appeals cautioned that:

> If [our prior decision stands] for the proposition that the disclosure must provide "guidance which will enable one skilled in the art to determine, *with reasonable certainty before performing the reaction,* whether the claimed product will be obtained,... then *all* "experimentation" is "undue," since the term "experimentation" implies that the success of the particular activity is *uncertain*. Such a proposition is contrary to the basic policy of the patent act...."

In re Angstadt, 537 F. 2d 498, 503, 190 USPQ 214, 218-219 (CCPA 1976). The court went on to emphasize that "the key word is "undue," not "experimentation." *Id* at 504, 190 USPQ at 219.

The court has further stated that "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (quoting *In re Jackson*, 217 USPQ 804, 807 (CCPA 1982)). In *Wands*, claims to antibodies that required a screening procedure to isolate the desired hybridoma cells from an enormous number of other cells present in the reaction mixture was held to not require experimentation that was "undue." *Id.* at 1406. The amount of effort required to make the antibodies was "not excessive." *Id.* at 1407. Similarly, applicants submit that the experimentation needed in the case of the presently claimed invention is not only routine in the art but that the instant specification provides more than sufficient guidance with respect to the direction in which the experimentation should proceed. This guidance is found in the present specification, at least on page 9, lines 19-30 and on page 30, line 20, through page 33, line 32.

One of skill in the art knows that miRNAs are small RNAs (typically 20-24 nucleotides long) that are processed from a precursor transcript in which the mature miRNA exists as part of an imperfect RNA duplex within the characteristic stem-loop structure of the precursor. Not all small RNAs meet this criterium and therefore, only a subset of small RNAs are defined as miRNAs. However, any small RNA that meets this criterium is, by definition, an miRNA. Thus, miRNAs are defined by the structure of the precursor from which they derive. This definition of miRNAs was well

known in the art at the time the present application was filed. (See, for example, Reinhart et al. (*Genes & Development* 16:1616-1626 (2002)) and Llave et al. (*Plant Cell* 14: 1605-1619 (2002)) (copies provided previously)).

Further, as discussed above, the presently claimed invention uses the well-known technique of compensating nucleotide base changes to modify the primary nucleotide composition of the miRNA nucleotide sequence while retaining any secondary structure that is present in the original miRNA precursor nucleotide sequence. Since the structure of the precursor is what defines an miRNA, the alteration of the primary sequence of the miRNA and use of compensating base changes to maintain the characteristic secondary stem-loop structure of the precursor, as described in the present specification, is a strategy that extends, by definition, to all miRNA genes.

The Action states that designing an artificial or non-natural miRNA precursor requires use of computational rules, such as thermodynamic profiling to predict an miRNA precursor molecule with the most favorable structure in solution. Action, pages 4-5. The Action cites to Krol et al. (*J. Biol. Chem.* 279: 42230-42239 (2004)) as teaching the relevance of free energy rules in designing an artificial miRNA precursor molecule. *Id.* The Action further states that the teachings of Krol et al. imply that random sequence mismatches with the miRNA sequence of an artificial miRNA precursor design would influence the free energy of the precursor molecule and which may negatively impact biogenesis. Action, page 5.

As an initial point, the present invention alters only the miRNA and the strand opposite the miRNA (hereinafter "miRNA*") and does so while maintaining the secondary structure of the miRNA precursor including mismatches. Thus, the present invention does not introduce "random" mismatches as alleged in the Action.

Furthermore, Krol et al. is concerned with the correctness of a predicted miRNA precursor structure (using computer analysis such as MFOLD) as compared to an experimentally determined structure and at most indicates only that predicted

and experimentally determined structures do not always agree. Krol et al. is discussed further in a Declaration under 37 C.F.R. § 1.132 of Dr. Bowman (hereinafter "the Bowman Declaration" provided herewith at Tab A), a co-inventor of this claimed invention. Dr. Bowman notes that the findings of Krol et al. indicate that it is relatively easier to predict the structure of the miRNA precursors when compared to most RNAs. Furthermore, as pointed out in the Bowman Declaration, the majority of the incorrect predictions in Krol et al. were found to occur in the terminal loop. Incorrect predictions in the terminal loop of the precursor are not relevant to the present application, since the present invention only describes altering the miRNA and miRNA* sequences.

On the basis of the disclosure in Krol et al., Dr. Bowman concludes that overall MFOLD does an excellent job predicting the structure of the miRNA precursor, especially in the miRNA-miRNA* region. Krol et al. shows that the structures of the miRNA precursors are quite predictable (88.4% of base pairs are correctly predicted) and when specifically discussing miRNA-miRNA* region, the percent correctly predicted is even higher (*See,* Krol et al., Fig. 2).

Furthermore, as discussed in the previous response submitted on January 3, 2007 (paragraph bridging pages 8-9) and in paragraph six of Dr. Vance's Declaration also submitted on January 3, 2007 (hereinafter "the Vance Declaration"), operable embodiments can be easily distinguished from inoperable embodiments by an assay that was routine in the art at the time of filing of the present application. Therefore, contrary to the assertions in the Action, one of skill in the art would view Krol et al. as supportive of the present invention as claimed and as described in the specification.

The Action further states that it is important to note that the bulges of a miRNA precursor play an important role in the overall recognition and processing of miRNA precursors and cites Alvarez et al. (*Plant Cell* 18: 1134-1151 (2006)) for this contention. Action, page 5. Applicants agree. The present specification specifically states that "bulges will be included and these too will be taken from the context of the endogenous precursor" (*See,* Specification, page 32, lines 22-23). As set forth in

the Bowman Declaration (and the Vance Declaration), Alvarez et al. is simply a further demonstration that the claimed invention works as described in the present specification. Thus, the disclosure in Alvarez et al. also supports the present invention as claimed and as described in the specification.

The Action concedes that the instant specification provides guidance on introducing mismatches in the non-native miRNA at the same sites as the native miRNA so that the newly designed miRNA precursors mimics the secondary structure of the naturally occurring miRNA precursor. Action, page 6. However, the Action further states that the instant claims encompass replacing a native miRNA sequence with any non-native nucleotide sequence which is complementary to a target sequence of interest but that the specification does not provide guidance on designing an artificial miRNA precursor of any length or of any base composition. *Id.* The Action concludes that in view of the claim breadth, one skilled in the art would not know where else mismatches can be tolerated within the non-native miRNA design without undue experimentation. *Id.*

As described above, claims 20 and 23 as presented herein recite that the modifications to the miRNA maintain the length of the endogenous miRNA and that the modifications to the miRNA precursor maintain the secondary structure of the miRNA precursor including double strandedness and any mismatches. The amendments to these claims clarify and further define the miRNA precursor constructs of the invention and applicants respectfully submit that they address the concerns raised in the Action as mentioned in the above paragraph.

Finally, the Action states that plant miRNA genes are known to exist in clusters and cites Mica et al. (*J. Exptl. Biol.* 57: 2601-2612 92006)) for support. Action, page 6. The Action then cites Lee et al. (*EMBO J.* 20: 4663-4670 (2002)), stating the Lee et al. teaches that a single promoter may drive transcription of the clustered miRNA genes to produce nascent polycistronic transcripts, which would require additional unknown processing steps to produce pre-miRNAs. *Id.* The Action concludes that undue experimentation would have been required at the time

the invention was made to determine how to design a plant miRNA precursor derived from clustered miRNA genes and comprising replacing a miRNA sequence endogenous to said plant miRNA precursor with a non-native miRNA sequence, so that the artificial miRNA precursor molecule is capable of undergoing normal biogenesis upon its expression in a plant. Action, page 7. Applicants respectfully disagree.

Mica et al. simply characterizes five conserved miRNA families in maize with respect to their expression pattern, identification of putative targets and chromosomal location. Mica et al. appears to be cited only for its mention of certain plants having miRNA genes organized into clusters.

With regard to Lee et al., as the Bowman Declaration describes, Lee et al., in fact, shows that the excision of multiple pre-miRNAs from a polycistronic precursor containing more than one pre-miRNA precursor sequence is no different than the excision of a single miRNA precursor sequence. Thus, miRNA precursors in polycistronic transcripts are fully capable of being used with the present invention, as are miRNA precursors that occur singly. In fact, as pointed out in both the Bowman Declaration and the Vance Declaration, using the protocol as described in the present application, Nui et al. (*Nat Biotechnol.* 24(11):1420-8 (2006)) has already demonstrated that such a "polycistronic" construct would work. Nui et al. constructed a dimeric gene, which contained the two individual artificial miRNA constructs ligated together and under control of the same promoter. Using this construct, Nui et al. were able to produce two different artificial miRNAs in a coordinated manner.

Finally, applicants emphasize that they have provided numerous examples that show that when a plant miRNA precursor construct is made and used as described in the present specification, it is processed properly to produce the modified miRNA and functions to reduce expression of the targeted gene as expected (e.g., Vaucheret et al., *Genes & Development* 18: 1187-1197 (2004); Schwab et al., *Plant Cell* 18: 1121-1133 (2006); Alvarez et al., *Plant Cell* 18: 1134-1151 (2006); and Nui et al., *Nat Biotechnol.* 24(11):1420-8 (2006); copies provided

previously). Additional examples evidencing that the present invention works as described are found in Choi et al. (*Development* 134: 1931-1941 (2007) and Qu et al. (*J. Virol.* 81: 6690-6699 (2007)) (copies of each of these references are attached at Tab C). No evidence has been presented to indicate that the claimed method would not work as described using any particular isolated plant miRNA precursor.

In a determination of whether the enablement requirement is satisfied the Examiner must consider all the evidence related to each of above eight factors and any conclusion of non-enablement must be based on the evidence as a whole. *In re Wands*, 858 F.2d, 731, at 737, 740, 8 USPQ 2d 1400, at 1404, 1407 (Fed. Cir. 1988).

When the evidence as a whole is considered, applicants respectfully submit that the claimed invention does not require undue experimentation, and thus claims 20, 23 and 26 satisfy the requirement for enablement. Applicants therefore respectfully request that the rejection be withdrawn.

B. Written Description.

The Action states that claims 20, 23, and 26 stand rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to comply with the written description requirement. Action, page 10. Specifically, the Action states that applicants have failed to describe the undisclosed structures of their broadly claimed genus and that one skilled in the art cannot reliably predict these structures based on the disclosure of miRNA167 and miRNA 171 precursors. Action, page 11. Applicants respectfully traverse this rejection.

Applicants respectfully contend that the specification does provide a sufficient written description so that one skilled in the art would appreciate that the Applicant was in possession of the claimed invention at the time of filing. Adequate written support does not require that the application contain an exhaustive enumeration of all possible plant miRNA precursors. Such information is readily available in the art. Furthermore, the structure of miRNA precursors is known [*see, e.g.*, Reinhart et al.

(*Genes & Development* 16:1616-1626 (2002)) and Llave et al. (*Plant Cell* 14: 1605-1619 (2002))] and, in fact, is used to define the set of small RNAs that are classified as miRNAs.

The USPTO itself has cautioned that "[t]he absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. § 112, para. 1, for lack of adequate written description" (Revised Interim Written Description Training Examples, "Synopsis of Application of Written Description Guidelines"). The USPTO has also clearly stated that "[i]nformation which is well known in the art need not be described in detail in the specification." *Guidelines for Examination of Patent Applications Under the 35 USC 112 ¶1, "Written Description" Requirement,* Federal Register 66, p. 1105 col. 3 (Jan. 5, 2001) (hereinafter, "Written Description guidelines").

Furthermore, with regard to establishing a representative number of species of the genus of miRNA precursors, the Written Description Guidelines indicate that where there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the species. In the present invention, there is not substantial variation between members of the claimed genus, as all members of the miRNA precursor family share significant structural features, the determination of which is well known in the art and was so at the time of the filing of the present application. In fact, microRNA structure precursor is well known in the art and would be readily recognized by one of skill in the art. Indeed, miRNAs are defined as small RNAs that are included within a precursor with this structure and, therefore, all miRNAs, by definition, have this characteristic precursor structure. Thus, all members of the genus have specific structural characteristics by which they are defined and recognized.

Applicants also point out that the Written Description Guidelines state that "[d]escription of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. In the present invention, the claimed genus is readily identified

by the limitations set forth in the specification and it is apparent that individual support for each species of the genus is not required for one of ordinary skill in the art to identify a plant miRNA precursor of this invention.

Applicants also note that in the Revised Interim Written Description Guidelines Training Materials, the Decision Tree presented on page 9 for evaluating whether a genus in an original claim meets the written description requirement shows that a determination of what is a representative number of species "...depends on whether one of skill in the art would recognize that applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed or claimed." In the present invention, one of skill in the art would readily recognize that applicants were in possession of plant miRNA precursors as disclosed in the claims and as defined in the specification to have specific structural requirements. These structural characteristics of the claimed genus of plant miRNA precursors define the necessary common attributes or features of the elements possessed by all plant miRNA precursors and certainly possessed by members of the genus of the claimed invention. Therefore, the specification provides a description of all of the elements that are essential to the operation and function of the genus as presented in the claimed invention.

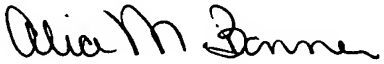
Thus, the claimed invention as a whole is readily identifiable, and any member of the claimed genus would be readily recognized by one of skill in the art. Accordingly, the skilled artisan would conclude that applicants were indeed in possession of the claimed invention as a whole. Therefore, the claimed invention meets the written description requirements of 35 U.S.C. § 112, first paragraph, and applicants respectfully request that this rejection be withdrawn.

Having addressed all of the issues raised by the Examiner in the pending Office Action, applicants believe that the claims as presented herein are in condition for allowance, which action is respectfully requested. The Examiner is invited and

encouraged to contact the undersigned directly if such contact will expedite the prosecution of the pending claims to issue.

The Commissioner is authorized to charge Deposit Account No. 50-0220 in the amount of $225.00 as the fee for a two-month extension of time (small entity). This amount is believed to be correct. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

Respectfully submitted,



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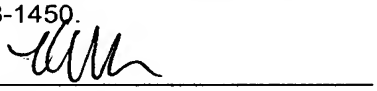

Katie Wu

Attorney Docket No. 9536-3 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Vance et al.
Application No.: 10/623,930
Filed: July 21, 2003
For: *Compositions and Methods for the Modulation of Gene Expression In Plants*

Confirmation No.: 6465
Group Art Unit: 1638
Examiner: V. Kumar

Date: August 20, 2007

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Tab A